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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: KroczeK

Application No.: To be assigned

Group Art Unit: To be assigned

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For: METHODS FOR TREATMENT OF
ASTHMATIC DISORDERS (as amended)

Attorney Docket No.: 7853-240

DECLARATION OF RICHARD KROCZEK UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, RICHARD KROCZEK do declare and state:

1. I am the inventor of the invention described and claimed in the above-identified patent application.
2. I presently hold the position of Professor of Molecular Immunology at the Robert Koch Institute, Berlin, Germany, the assignee of the above-identified patent application. My *curriculum vitae* is attached hereto as Exhibit 1.
3. I have read and am familiar with the instant application.
4. The invention claimed in the present application is directed to methods of treating asthmatic disorders comprising administering monoclonal antibodies directed against a human polypeptide, referred to in the present application as the "8F4 polypeptide." Since the original filing date of the specification of PCT Application PCT/DE98/02896, to which the present application claims priority, the 8F4 polypeptide has come to be referred to in the literature as "ICOS" (Inducible T cell Co-Stimulator). A polypeptide corresponding to an 8F4 polypeptide has also been referred to in the literature as "H4."

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5. Described in paragraphs 6 to 11 below is a study by Gonzalo *et al.* (2001, "ICOS is critical for T helper cell-mediated lung mucosal inflammatory responses," *Nat. Immunol.* 2(7):597-604, attached hereto as Exhibit 2)("Gonzalo"), of which I am a co-author, that demonstrates that administration of ICOS-inhibitory compounds, such an antibody that recognizes ICOS and an ICOS polypeptide, in an accepted experimental model of human asthma, result in abrogation of symptoms of the disease. Further described in paragraphs 12 to 14 below are experiments done by me or by others under my supervision that demonstrate that ICOS-expressing cells are associated with lung inflammation resulting in asthma in humans. These experiments corroborate the teaching provided in the present application by indicating that successful *in vivo* amelioration of asthma symptoms can be achieved by administration of antibodies that recognize the human ICOS polypeptide or the ICOS polypeptide itself.

**INHIBITION OF ASTHMA SYMPTOMS BY ICOS-
INHIBITORY MOLECULES IN A MOUSE MODEL OF ASTHMA**

6. The study reported by Gonzalo provides evidence of the critical role of ICOS in the pathology of asthma. The experiments described by Gonzalo utilize an art-recognized mouse model for asthma, DO11.10 (Wills-Karp, 2000, *Immunopharmacology* 48:263-268, attached hereto as Exhibit 3). DO11.10 transgenic mice express a T cell receptor specific for the OVA antigen (amino acid residues 323-339 of chicken ovalbumin). Briefly, upon exposure to particular antigens, DO11.10 mice exhibit symptoms (e.g., lung mucosal inflammation) that are characteristic of asthma in humans. The severity of the symptoms in such treated mice was notably reduced by administration of ICOS inhibitory molecules to the mice.

7. More specifically, exposure of DO11.10 mice to the OVA antigen results in the production of a variety of inflammation-promoting cytokines in the bronchioalveolar fluid (BAL) of the mice. In contrast, as described in the Section at page 600 entitled "ICOS- and CD28-mediated cytokine regulation" and the accompanying Figure 7, administration of a monoclonal antibody that recognizes ICOS, 12A8, prior to exposure of the mice to the OVA antigen reduces the levels of inflammatory cytokines in the BAL of the mice relative to untreated DO11.10 mice. Similarly, administration of a soluble form of ICOS polypeptide, ICOS-Ig, prior to exposure of the mice to the OVA antigen reduces the levels of inflammatory cytokines in the BAL of the mice relative to untreated DO11.10 mice. Thus, administration of a monoclonal antibody that recognizes ICOS or an ICOS polypeptide reduces a hallmark of asthma.

8. Another hallmark of asthma in humans is accumulation of lymphocytes and eosinophils in the BAL upon exposure to antigenic stimulus. After persistent exposure to OVA, DO11.10 mice exhibit such accumulation. As described in the Section on page 599 entitled "Regulation of mucosal inflammation by ICOS" and Figure 5, the administration of 12A8 antibody DO11.10 mice before each exposure to OVA reduces lymphocyte and eosinophil accumulation by 50 and 70%, respectively. The authors of Gonzalo further note, also as shown in Figure 5, that comparable suppression of lymphocyte and eosinophil accumulation observed upon administering ICOS-Ig. Again, this represents a reduction in a hallmark of asthma.

9. In summary, the experimental results presented in Gonzalo demonstrate that administration of ICOS inhibitory compounds, such as anti-ICOS antibodies or ICOS proteins, can ameliorate symptoms of asthma.

ICOS IN HUMAN ASTHMA

10. The results presented hereinbelow demonstrate that ICOS is, indeed, involved in human lung inflammation such as that seen in asthma. This human data further evidences that administration of ICOS inhibitory compounds to humans will mirror the effects of administering such compounds to the art accepted model of human asthma, and accordingly, that ICOS inhibitory compounds will be useful in treating human asthmatic disorders.

11. Airway hyperresponsiveness and pulmonary inflammation are the two central hallmarks of human allergic asthma. The inflammatory process is initiated and maintained by T cells. The crucial role of certain co-stimulatory pathways in allergic asthma has been demonstrated in mice and several studies in the human (Exhibits 4-5).

12. To assess the participation of the ICOS molecule in the pathogenesis of human asthma, individuals with mild asthma were exposed to allergen. The expression of ICOS on T cells present in the bronchoalveolar lavage (BAL) fluid collected 42 hours after segmental allergen provocation was analyzed by flow cytometry, as described in Exhibit 6. These data, described in Exhibit 7, were correlated with immunohistological studies on ICOS expression in bronchial biopsies taken at the time the performed BAL was performed.

13. The data clearly demonstrate that a very substantial proportion of the infiltrating T cells found in the submucosa and in the epithelium bear significant levels of ICOS. These ICOS-positive T cells transmigrate into the bronchial space on allergen exposure.

14. The finding that ICOS is involved in human lung inflammation validates the results obtained by Gonzalo demonstrating the inhibition of hallmarks of asthma by ICOS inhibitory molecules in a mouse model of asthma, and signifies that ICOS inhibitory compounds will have similar effects on human asthma. Thus, taken together, the human data presented herein and the mouse model data shown by Gonzalo strongly indicate that: a) ICOS participates in the pathogenesis of allergic asthma in humans; and b) amelioration of asthma symptoms in humans can be achieved by administration of antibodies that recognize the ICOS polypeptide or by administration of ICOS polypeptides.

15. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated:

October 4, 2001

Richard KroczeK

Attachments:

- Exhibit 1: *Curriculum Vitae* of Richard KroczeK
- Exhibit 2: Gonzalo *et al.*, 2001, "ICOS is critical for T helper cell-mediated lung mucosal inflammatory responses," *Nat. Immunol.* 2(7):597-604.
- Exhibit 3: Wills-Karp, 2000, *Immunopharmacology* 48:263-268.
- Exhibit 4: Keane-Myers *et al.*, 1998, "Development of murine allergic asthma is dependent upon B7-2 costimulation," *J. Immunol.* 160:1036-1043.
- Exhibit 5: Mathur *et al.*, 1999, "CD28 interaction with either CD80 or CD86 are sufficient to induce allergic airway inflammation in mice," *Am. J. Respir. Cell. Mol. Biol.* 21:498-509.
- Exhibit 6: Materials and Methods for Human Asthma Analysis
- Exhibit 7: Results for Human Asthma Analysis